AMENDMENT TO THE SPECIFICATION

Please replace the paragraph beginning on page 67, beginning at line 5 with the following paragraph:

The gene junctions of the genomes of paramyxoviruses contain short and highly conserved nucleotide sequences at the beginning and end of each gene (gene start and gene end signals), possibly playing a role in initiation and termination of transcription (Curran *et al.,* 1999). Comparing the intergenic sequences between all genes of MPV revealed a consensus sequence for the gene start signal of the N, P, M, F, M2 and G: GGGACAAGU (SEQ ID NO: 166) (Figure 17A), which is identical to the consensus gene start signal of the metapneumoviruses (Ling *et al.,* 1992; Yu *et al.,* 1992; Li *et al.,*1996; Bäyon-Auboyer *et al.,* 2000). The gene start signals for the SH and L genes of MPV were found to be slightly different from this consensus (SH: GGGAUAAAU, (SEQ ID NO: 167) L: GAGACAAAU). (SEQ ID NO: 168) For APV the gene start signal of L was also found to be different from the consensus: AGGACCAAT (SEQ ID NO: 169) (APV-A) (Randhawa *et al.,* 1996) and GGGACCAGT (SEQ ID NO: 170) (APV-D) (Bäyon-Auboyer *et al.,* 2000).

In contrast to the similar gene start sequences of MPV and APV, the consensus gene end sequence of APV, UAGUUAAUU (SEQ ID NO: 171) (Randhawa *et al.,* 1996), could not be found in the MPV intergenic sequences. The repeated sequence found in most genes, except the G-L intergenic region, was U AAAAA U/AC, which could possibly act as gene end signal. However, since we sequenced viral RNA rather than mRNA, definitive gene end signals could not be assigned and thus requires further investigation. The intergenic regions of pneumoviruses vary in size and sequence (Curran *et al.,* 1999; Blumberg *et al.,* 1991; Collins et al., 1983;). The intergenic regions of MPV did not reveal homology with those of APV and RSV and range in size from 10 to 228 nucleotides (Figure 17B). The intergenic region between the M and F ORFs of MPV contains part of a secondary ORF, which starts in the primary M ORF (see above).

The intergenic region between SH and G contains 192 nucleotides, and does not appear to have coding potential based on the presence of numerous stop-codons in all three reading frames. The intergenic region between G and L contains 241 nucleotides, which may include additional ORFs (see above). Interestingly, the start of the L ORF is located in these secondary ORFs. Whereas the L gene of APV does not start in the preceding G ORF, the L ORF of RSV also starts in the preceding M2 gene. At the 3' and 5' extremities of the genome of paramyxoviruses short extragenic region are referred to as the leader and trailer sequences,

and approximately the first 12 nucleotides of the leader and last 12 nucleotides of the trailer are complementary, probably because they each contain basic elements of the viral promoter (Curran *et al.,* 1999; Blumberg *et al.,* 1991; Mink *et al.,* 1986). The 3'leader of MPV and APV are both 41 nucleotides in length, and some homology is seen in the region between nucleotide 16 and 41 of both viruses (18 out of 26 nucleotides) (Figure 17B). As mentioned before the first 15 nucleotides of the MPV genomic map are based on a primer sequence based on the APV genome. The length of the 5'trailer of MPV (188 nucleotides) resembles the size of the RSV 5'trailer (155 nucleotides), which is considerably longer than that of APV (40 nucleotides). Alignments of the extreme 40 nucleotides of the trailer of MPV and the trailer of APV revealed 21 out of 32 nucleotides homology, apart from the extreme 12 nucleotides which represent primer sequences based on the genomic sequence of APV. Our sequence analyses revealed the absence of NS1 and NS2 genes at the 3'end of the genome and a genomic organisation resembling the organisation of metapneumoviruses (3'-N-P-M-F-M2-SH-G-L-5'). The high sequence homology found between MPV and APV genes further emphasises the close relationship between these two viruses. For the N, P, M, F, M2-1 and M2-2 genes of MPV an overall amino acid homology of 79% is found with APV-C. In fact, for these genes APV-C and MPV revealed sequence homologies which are in the same range as sequence homologies found between subgroups of other genera, such as RSV- A and B or APV-A and B. This close relationship between APV-C and MPV is also seen in the phylogenetic analyses which revealed MPV and APV-C always in the same branch, separate from the branch containing APV-A and B. The identical genomic organisation, the sequence homologies and phylogentic analyses are all in favour of the classification of MPV as the first member in the *Metapneumovirus* genus that is isolatable from mammals. It should be noted that the found sequence variation between different virus isolates of MPV in the N, M, F and L genes revealed the possible existence of different genotypes (van den Hoogen *et al.,* 2001). The close relationship between MPV and APV-C is not reflected in the host range, since APV infects birds in contrast to MPV (van den Hoogen *et al.,* 2001). This difference in host range may be determined by the differences between the SH and G proteins of both viruses that are highly divergent. The SH and G proteins of MPV did not reveal significant aa sequence homology with SH and G proteins of any other virus. Although the amino acid content and hydrophobicity plots are in favour of defining these ORFs as SH and G, experimental data are required to assess their function. Such analyses will also shed light on the role of the additional overlapping ORFs in these SH and G genes. In addition, sequence

analyses on the SH and G genes of APV-C might provide more insight in the function of the SH and G proteins of MPV and their relationship with those of APV-C. The noncoding regions of MPV were found to be fairly similar to those of APV. The 3'leader and 5' trailer sequences of APV and MPV displayed a high degree of homology. Although the lengths of the intergenic regions were not always the same for APV and MPV, the consensus gene start signals of most of the ORFs were found to be identical. In contrast, the gene end signals of APV were not found in the MPV genome. Although we did find a repetitive sequence (U AAAAA U/A/C) (SEQ ID NO: 172) in most intergenic regions, sequence analysis of viral mRNAs is required to formally delineate those gene end sequences. It should be noted that sequence information for 15 nucleotides at the extreme 3'end and 12 nucleotides at the extreme 5'end is obtained by using modified rapid amplification of cDNA ends (RACE) procedures. This technique has been proven to be successful by others for related viruses (Randhawa, J.S. et al., Rescue of synthetic minireplicons establishes the absence of the NS1 and NS2 genes from avian pneumovirus. J. Virol, 71, 9849-9854 (1997); Mink, M.A., et al. Nucleotide sequences of the 3' leader and 5' trailer regions of human respiratory syncytial virus genomic RNA. Virology 185, 615-24 (1991).) To determine the sequence of the 3' vRNA leader sequence, a homopolymer A tail is added to purified vRNA using poly-A-polymerase and the leader sequence subsequently amplified by PCR using a poly-T primer and a primer in the N gene. To determine the sequence of the 5' vRNA trailer sequence, a cDNA copy of the trailer sequence is made using reverse transcriptase and a primer in the L gene, followed by homopolymer dG tailing of the cDNA with terminal transferase. Subsequently, the trailer region is amplified using a poly-C primer and a primer in the L gene. As an alternative strategy, vRNA is ligated to itself or synthetic linkers, after which the leader and trailer regions are amplified using primers in the L and N genes and linker-specific primers. For the 5' trailer sequence direct dideoxynucleotide sequencing of purified vRNA is also feasible (Randhawa, 1997). Using these approaches, we can analyse the exact sequence of the ends of the hMPV genome. The sequence information provided here is of importance for the generation of diagnostic tests, vaccines and antivirals for MPV and MPV infections.

IN THE SEQUENCE LISTING

Please enter the Substitute Sequence Listing enclosed herewith into the application.